

# Changes of Seed Weight, Fatty Acid Composition, and Oil and Protein Contents from Different Peanut *FAD2* Genotypes at Different Seed Developmental and Maturation Stages

Ming Li Wang,<sup>\*,†,‡</sup> Charles Y. Chen,<sup>‡</sup> Brandon Tonniss,<sup>†</sup> David Pinnow,<sup>†</sup> Jerry Davis,<sup>§</sup> Yong-Qiang Charles An,<sup>||</sup> and Phat Dang<sup>⊥</sup>

<sup>†</sup>Plant Genetic Resources Conservation Unit, Agricultural Research Service (ARS), United States Department of Agriculture (USDA), Griffin, Georgia 30223, United States

<sup>‡</sup>Department of Crop, Soil and Environmental Sciences, Auburn University, Auburn, Alabama 36849, United States

<sup>§</sup>Department of Experimental Statistics, University of Georgia, Griffin, Georgia 30223, United States

<sup>||</sup>Plant Genetics Research Unit, Agricultural Research Service (ARS), United States Department of Agriculture (USDA), Donald Danforth Plant Science Center, St. Louis, Missouri 63132, United States

<sup>⊥</sup>National Peanut Research Laboratory, Agricultural Research Service (ARS), United States Department of Agriculture (USDA), Dawson, Georgia 39842, United States

**ABSTRACT:** The level of oleic acid in peanut seed is one of the most important factors in determining seed quality and is controlled by two pairs of homeologous genes (*FAD2A* and *FAD2B*). The genotypes of eight  $F_8$  breeding lines were determined as *AABB*, *aaBB*, *AAbb*, and *aabb* by real-time polymerase chain reaction and sequencing. Fresh seeds were collected from five seed developmental stages and, after drying, were used for chemical analysis. Our results showed that (1) as seeds developed, seed weight, oil content, and oleic acid level significantly increased, whereas four other fatty acid levels decreased, but protein content and another four fatty acid levels did not significantly change, (2) *FAD2A/FAD2B* significantly affected fatty acid profiles but not oil and protein contents, and (3) the data were consistent across 2 years. The variability of seed quality traits revealed here will be useful for peanut breeders, farmers, processors, and consumers.

**KEYWORDS:** *Arachis hypogaea*, cultivated peanut, fatty acid desaturase 2 (*FAD2*) genotype, seed developmental stage, seed chemical composition, nuclear magnetic resonance (NMR), nitrogen analyzer (NA), gas chromatography (GC)

## INTRODUCTION

Peanut is one of the most important oilseed crops worldwide. Peanut seeds contain about 50% oil, 25% protein, 15% carbohydrates, 2% fiber, 2% ash, and 6% water. They also contain minor bioactive compounds, such as folates, minerals, vitamin E, resveratrol, and flavonoids, which have antioxidant activities. Because oil is the major component of peanut seeds, the fatty acid composition is a key factor for determining the seed quality. Oleic acid (monounsaturated acid, C18:1) is a major fatty acid in cultivated peanut seeds, with an average of 45%, ranging from 31 to 82% (unpublished data from screening 8800 accessions in the U.S. cultivated peanut collection). Consuming peanut oil containing about 80% oleic acid (similar to olive oil containing about 75% oleic acid) can benefit human health by lowering the cardiovascular disease (CVD) risk by 15%.<sup>1</sup> Peanut seeds containing a high level of oleic acid (monounsaturated acid) and a low level of linoleic acid (polyunsaturated acid) are less susceptible to rancidification, resulting in a longer shelf life of stored peanut or peanut products. Therefore, developing peanut cultivars containing high oleic acid is one of the main objectives in peanut breeding programs.

In most peanut cultivars (*Arachis hypogaea*,  $2n = 4x = 40$ , *AABB*), the oleic acid level is mainly controlled by two pairs of homeologous genes. These two genes encoding fatty acid desaturase (FAD) enzymes are located on genomes A and B and are designated

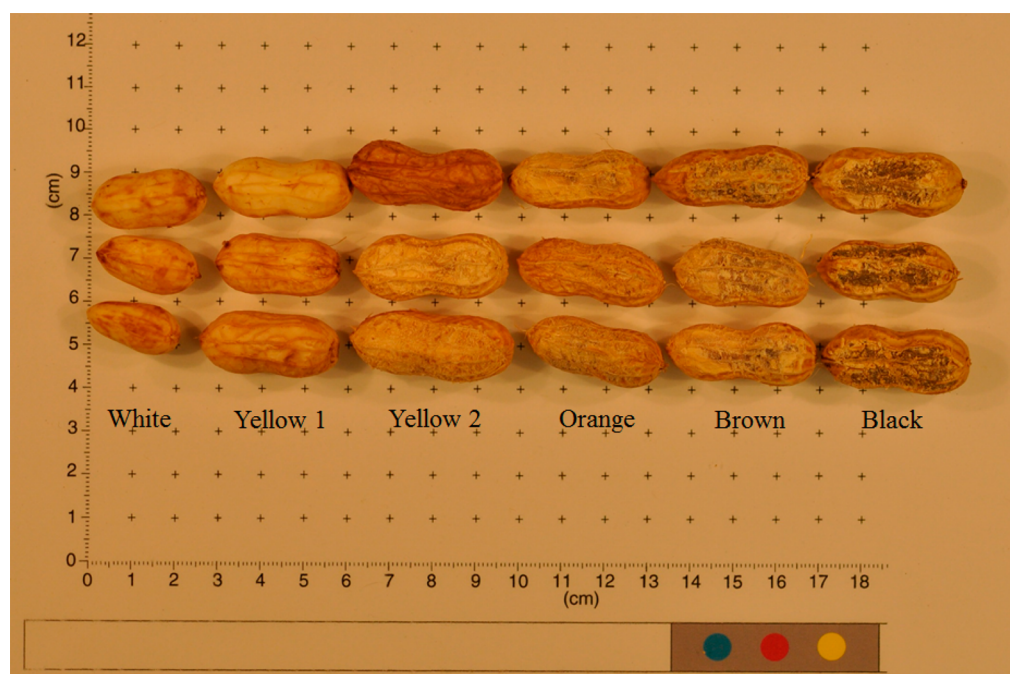
*FAD2A* and *FAD2B*, respectively.<sup>2,3</sup> The FAD enzyme converts oleic acid (C18:1) into linoleic acid (C18:2). The functional mutations in the genes for the enzyme can greatly reduce FAD activity, which causes the levels of oleic and linoleic acids to increase and decrease, respectively.<sup>3,4</sup> Sequence analysis of *FAD2A* and *FAD2B* genes in the double mutant identified a base substitution G448A on genome A and an insertion 442insA on genome B, leading to D150N (a missense amino acid substitution from aspartic acid to asparagine) and a premature stop codon, respectively. On the basis of these mutations, there are four homozygous genotypes identified: wild type (*AABB*, no mutation on *FAD2A* and *FD2B*), a single mutation on *FAD2A* (*aaBB*) on genome A, a single mutation on *FAD2B* (*AAbb*) on genome B, and a double mutation on both *FAD2A* and *FAD2B* (*aabb*). Thus far, there have been no mutants identified naturally that only contained a single functional mutation on *FAD2B* but normal function on *FAD2A*. All of the single mutants (*AAbb*) on *FAD2B* were created by the selection of segregation progenies from the crosses generated between high oleic acid parent (*aabb*) and wild type (*AABB*). Different *FAD2* genotypes can significantly affect the level of oleic acid in peanut seed.

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**Figure 1.** Peanut pod development stages and peanut seeds collected from different stages.

Peanut is one of the unique legume species producing pods underground and, thus, also called “groundnut”. When the plant is reaching harvest time (physiological maturity stage), different pods harvested from the same plant can be at different seed developmental and maturation stages. On the basis of pod colors, seed development and maturation were described as 14 stages.<sup>5</sup> However, for seed collection purposes, a Peanut Profile Board [jointly described by the Agricultural Research Service (ARS), United States Department of Agriculture (USDA), the Georgia Peanut Commission, and the University of Georgia], classifies pods into six developmental and maturation stages (white, yellow 1, yellow 2, orange, brown, and black). In early reports,<sup>6–8</sup> some seed quality characters (such as sugars, starch, and fatty acid composition) at different developmental stages had been documented, but comprehensive and in-depth characterization of seed chemical composition and its dynamic changes in different *FAD2* genotypes over the course of seed developmental and maturation stages is not available. This information will be very useful for peanut breeders to determine their selection strategies, peanut farmers to determine their harvesting time, food processors to determine their chosen seed types in food production, and consumers to choose their preferred nutritional products.

To fill this knowledge gap, we grew four peanut *FAD2* genotypes for 2 years. After harvesting and high-pressure blasting the pod skin, the peanut pods were sorted into five seed developmental and maturation stages. The seeds from different developmental stages were used for chemical analysis. Therefore, the objectives of this study were to (i) determine the changes in seed oil and protein contents, seed weight, and fatty acid composition during seed development and maturation, (ii) compare the effect of genotype (*AABB*, *aaBB*, *Aabb*, and *aabb*) in *FAD2A/FAD2B* genes on seed oil and protein contents, seed weight, and fatty acid composition, (iii) determine the correlations among investigated seed quality traits, and (iv) determine whether enhancing the level of oleic acid can significantly reduce the oil and protein contents.

## MATERIALS AND METHODS

### Selection of Four Genotypes by Real-Time Polymerase Chain Reaction (PCR) and Sequence Analysis of *FAD2A/FAD2B* Genes.

The breeding lines of progeny  $F_3$  for developing high oleic acid cultivars from the peanut breeding program at Auburn University were first screened by genotyping using real-time PCR. On the basis of the field agronomic performance and real-time PCR screening results, eight lines were selected. Then, *FAD2A/FAD2B* genes from these eight lines were amplified by PCR and sequenced. On the basis of the sequence results, these eight lines were classified into four genotypes: *AA/BB*, *aa/BB*, *AA/bb*, and *aa/bb*. Lines 1 and 2 belonged to *AA/BB* genotype. Lines 3 and 4 belonged to *aa/BB* genotype. Lines 5 and 6 belonged to *AA/bb* genotype. Lines 7 and 8 belonged to *aa/bb* genotype.

### Collection of Peanut Pods under Different Developmental and Maturation Stages.

A total of 10 seeds from each of these eight lines were planted in environmentally controlled plots at the National Peanut Research Laboratory, ARS, USDA, for 2 years (2015 and 2016). The soil type consisted of a Greenville sandy clay loam (fine, kaolinitic, thermic Rhodic Kandiudults). During growth, plants were fully irrigated on the basis of soil moisture sensor measurements (MPS-2, Decagon Devices, Pullman, WA, U.S.A.). Fungicides, herbicides, insecticides, and soil nutrition supplements were applied throughout the growing season in accordance with recommended production practices. Single plants were dug out, and pods were harvested by hand. Peanut pods were pressure-blasted using a lightweight pressure washer (Troy-Bilt 2800-PSI max) with a turbo nozzle attachment and sorted into six seed progressive developmental stages (white, yellow 1, yellow 2, orange, brown, and black; see Figure 1) based on a modified method originally described as the hull-scrub method.<sup>5</sup> Peanut pods were subjected to mechanical curing until the kernel moisture was around 10%, using heated air at 8 °C above ambient temperature.<sup>9</sup> After drying, peanut pods at five different developmental and maturation stages (the white stage was excluded because not enough seeds were collected) were shelled by hand and used for chemical analysis.

**Chemical Analysis of Seed Quality Traits.** In total, 11 chemical traits were investigated, including seed oil and protein contents, nine fatty acids, and also 100 seed weight.

**Oil Content by Nuclear Magnetic Resonance (NMR) Analysis.** The oil content was quantified by NMR analysis on a minispec seed analyzer (Bruker Optics, Inc., Houston, TX, U.S.A.) following the standard operating procedure. Seed oil and water contents were measured, and

Table 1. Variability in Investigated Seed Quality Traits<sup>a</sup>

trait	n	mean	SD	minimum	maximum
protein (%)	80	23.58	1.57	19.84	27.19
oil (%)	77	47.73	3.51	37.04	53.70
seed weight (g)	80	46.73	16.66	19.32	83.98
C16:0 (%)	80	10.41	2.70	5.70	14.45
C18:0 (%)	80	1.97	0.37	1.15	3.04
C18:1 (%)	80	54.45	15.99	34.49	81.81
C18:2 (%)	80	26.08	13.78	2.40	43.38
C20:0 (%)	80	1.12	0.13	0.83	1.51
C20:1 (%)	80	1.55	0.43	0.82	2.67
C22:0 (%)	80	2.79	0.49	1.92	4.02
C24:0 (%)	80	1.57	0.39	0.87	2.70
C26:0 (%)	80	0.18	0.14	0.00	0.41

<sup>a</sup>Standard deviation (SD), seed protein, oil, and methyl esters (%), and seed weight (g/100 seeds).

the mass of each measurement was converted to a percentage of the total weight of each sample. All samples were measured in triplicate, and the results were averaged.

**Fatty Acid Composition by Gas Chromatography (GC) Analysis.** Fatty acid methyl esters (FAMES) were prepared from seeds by alkaline transmethylation,<sup>10</sup> and fatty acid composition was determined using an Agilent 7890A GC equipped with a flame ionization detector (FID) and an autosampler. Sample preparation, GC operation, and data collection followed the standard methods used by our lab routinely.<sup>11</sup>

**Protein Content by Nitrogen Analyzer.** The seed protein content was measured using a rapid N exceed nitrogen analyzer (Elementar, Hanau, Germany). The sample preparation and measurement procedure followed the protocol developed by our laboratory.<sup>12</sup> The total nitrogen content was expressed as a percentage of the sample mass. A protein factor of 5.46 was used to estimate the protein content in peanut seeds (protein % = N % × 5.46).

**Seed Weight.** In addition to 11 investigated chemical traits, seed weight was also measured for each line. Three samples of available seeds from each line were counted and weighed. The average weight for each genotype and stage was expressed as grams per 100 seeds.

**Statistical Analysis.** Data were recorded by replicates, five developmental stages, and four genotypes for 2 years. An analysis of variance was performed on the data, and means were separated using Tukey's multiple comparison procedure (SAS Online Doc 9.2, SAS Institute, Inc., Cary, NC, U.S.A.). Correlations between investigated seed traits were determined using Pearson correlation coefficients.

## RESULTS AND DISCUSSION

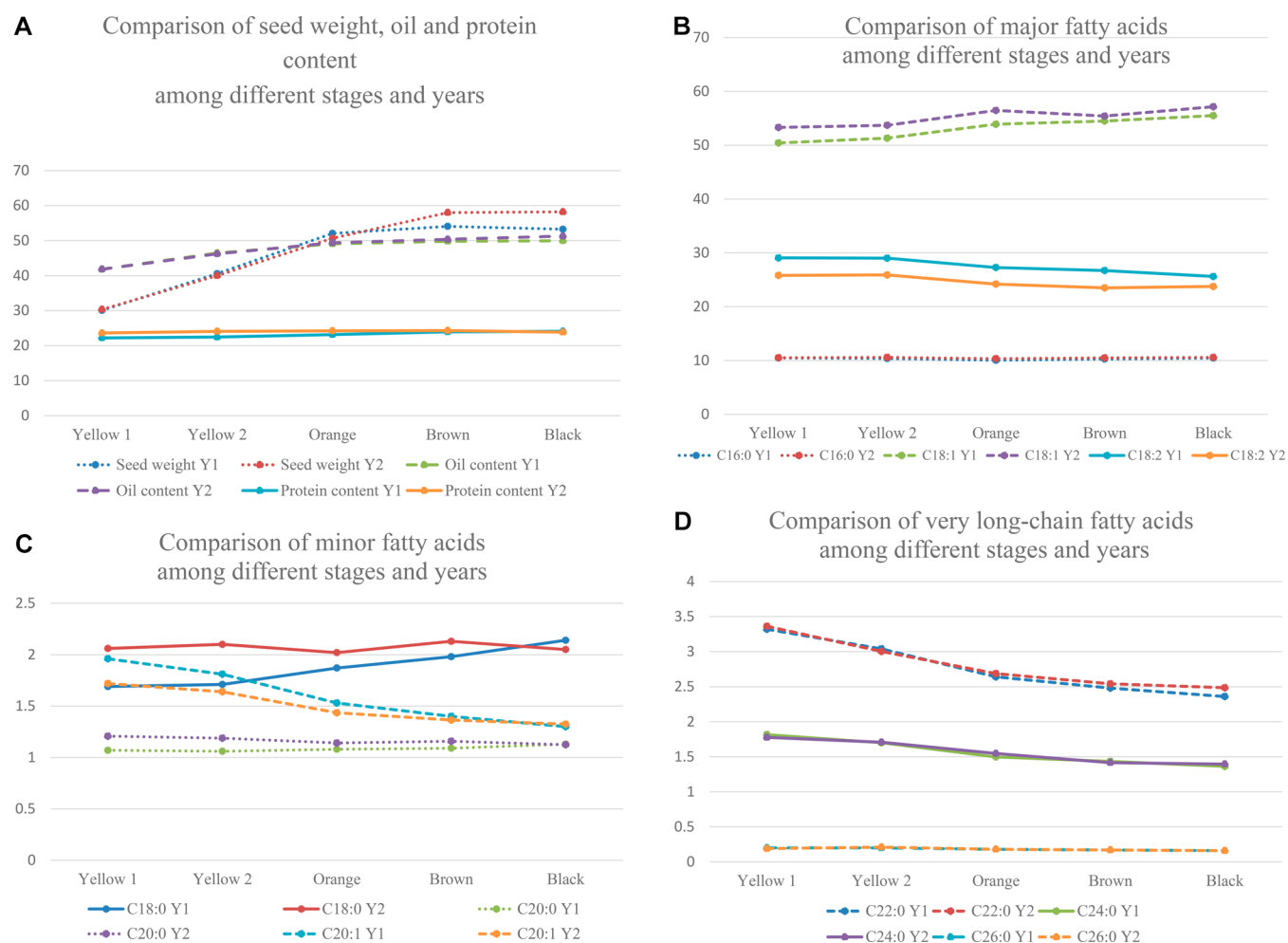
### Variability of the Investigated Seed Quality Traits.

Significant variability was identified in seed quality traits among different *FAD2* genotypes from seeds collected from different seed developmental and maturation stages (Table 1). There was significant variation in 100 seed weight, with an average of 46.73 g, ranging from 19.32 to 83.98 g. There was also significant variation in protein and oil contents, with averages of 23.58 and 47.73% (ranging from 19.84 to 27.19% and from 37.04 to 53.7%), respectively. The oil content had a wider range of variation than the protein content. There was significant variation in major fatty acids. Variation in palmitic acid (C16:0) ranged from 5.7 to 14.45%, with an average 10.41%. Variation in oleic acid (C18:1) and linoleic acid (C18:2) ranged from 34.49 to 81.81% and from 2.4 to 43.38%, with an average of 54.45 and 26.08%, respectively. Linoleic acid had a much wider variation than oleic acid. There was significant variation in minor fatty acids. Variation in stearic acid (C18:0) ranged from 1.15 to 3.04%, with an average of 1.97%. Variation in arachidic acid (C20:0) and gadoleic acid (C20:1) ranged from 0.83 to 1.51% and from 0.82 to 2.67%, with an average of 1.12 and 1.55%, respectively. There was significant variation in very long-chain ( $C \geq 22$ ) fatty acids. Variation in

Table 2. Comparison of Seed Chemical Composition among Five Developmental Stages<sup>a</sup>

trait	year	yellow 1	yellow 2	orange	brown	black	MSD	range	average
protein (%)	1	22.20 a	22.44 a	23.14 a	23.93 a	24.10 a	3.19	22.20	21.58
	2	23.60 a	24.08 a	24.20 a	24.30 a	23.84 a	1.15	24.30	
oil (%)	1	41.84 c	46.46 b	49.08 ab	49.82 a	49.95 a	2.81	41.84	47.72
	2	42.81 d	46.23 c	49.38 b	50.37 ab	51.29 a	1.48	51.29	
seed weight (g)	1	30.09 c	40.56 b	52.06 a	53.26 a	54.08 a	9.31	30.09	46.73
	2	30.34 c	40.00 bc	50.66 ab	58.01 a	58.22 a	13.13	58.22	
C16:0 (%)	1	10.47 a	10.36 a	10.04 a	10.27 a	10.45 a	0.44	10.04	10.41
	2	10.52 a	10.59 a	10.35 a	10.50 a	10.58 a	0.76	10.59	
C18:0 (%)	1	1.69 c	1.71 c	1.87 bc	1.98 ab	2.14 a	0.27	1.69	1.98
	2	2.06 a	2.10 a	2.02 a	2.13 a	2.05 a	0.24	2.14	
C18:1 (%)	1	50.43 c	51.14 bc	53.90 ab	54.50 ab	55.51 a	3.46	50.43	54.15
	2	53.31 c	53.70 bc	56.46 ab	55.42 a	57.16 a	3.12	57.16	
C18:2 (%)	1	29.07 a	29.01 a	27.28 ab	26.71 ab	25.61 b	2.86	23.50	26.08
	2	25.82 a	25.89 a	24.20 a	23.50 a	23.75 a	3.40	29.01	
C20:0 (%)	1	1.07 a	1.06 a	1.08 a	1.09 a	1.13 a	0.07	1.06	1.13
	2	1.21 a	1.19 a	1.14 a	1.16 a	1.12 a	0.09	1.21	
C20:1 (%)	1	1.96 a	1.81 ab	1.53 bc	1.40 c	1.30 c	0.35	1.30	1.55
	2	1.72 a	1.64 a	1.44 b	1.36 b	1.32 b	0.14	1.96	
C22:0 (%)	1	3.32 a	3.04 ab	2.64 bc	2.48 c	2.36 c	0.41	2.36	2.79
	2	3.36 a	3.00 b	2.69 c	2.54 cd	2.49 d	0.20	3.36	
C24:0 (%)	1	1.82 a	1.70 ab	1.50 ab	1.43 bc	1.36 c	0.32	1.36	1.57
	2	1.78 a	1.71 a	1.55 b	1.42 c	1.40 c	0.12	1.82	
C26:0 (%)	1	0.20 a	0.20 a	0.18 a	0.17 a	0.16 a	0.10	0.16	0.18
	2	0.19 a	0.21 a	0.18 a	0.17 a	0.16 a	0.05	0.21	

<sup>a</sup>Five developmental stages: yellow 1, yellow 2, orange, brown, and black. Minimum significant difference (MSD), seed protein, oil, and methyl esters (%), and seed weight (g/100 seeds). If following with the same letter after the trait value in each row, there were no statistically significant differences between two or more stages.



**Figure 2.** Comparison of seed quality traits among five seed developmental and maturation stages for 2 years: (A) comparison of seed weight and oil and protein contents, (B) comparison of major fatty acids, (C) comparison of minor fatty acids, and (D) comparison of very long-chain fatty acids.

behenic acid (C22:0) ranged from 1.92 to 4.02%, with an average of 2.79%. Variation in lignoceric acid (C24:0) and hexacosanoic acid (C26:0) ranged from 0.87 to 2.70% and from 0 to 0.41%, with an average of 1.57 and 0.18%, respectively. Peanut seeds contained ~5% very long-chain saturated fatty acids, but their biological function is still not clear.<sup>1</sup>

**Changes of Seed Weight, Oil and Protein Contents, and Fatty Acid Composition at Five Different Seed Developmental and Maturation Stages.** The results of variability in seed quality traits from five seed developmental and maturation stages are listed in Table 2 and shown in Figure 2. As seed development proceeded (from yellow 1 to black), the oil content and seed weight significantly increased from 41.84 to 51.29% and from 30.09 to 58.22 g/100 seeds, respectively. However, changes in the protein content (22–24%) from yellow 1 to black stages were not statistically significant. In general, the protein and oil contents and seed weight results from 2 years were consistent (Table 2 and Figure 2A). For major fatty acids, the oleic acid level significantly increased (50.53–57.16%), whereas the linoleic acid level significantly decreased (29.07–23.75%), but the palmitic acid level remained consistent (10.47–10.59%) from yellow 1 to black stages. The results from 2 years were also consistent. During seed development and maturation, as fresh seed weight increased, the level of oleic acid increased and the level of linoleic acid decreased. This similar trend was also observed in other peanut studies.<sup>13</sup> For minor fatty acids, the level of gadoleic acid (C20:1)

significantly decreased (from 1.96 to 1.30%) but the level of arachidic acid (C20:0) did not have a significant change (from 1.06 to 1.21%). There was a difference in the level of stearic acid between the 2 years. For the first year, the level of stearic acid (1.69–2.14%) increased, but its level from the second year did not have a significant change (2.02–2.10%). For very long-chain fatty acids, both levels of behenic acid (C22:0) and lignoceric acid (C24:0) significantly decreased (3.36–2.36 and 1.82–1.36%) but the level of hexacosanoic acid (C26:0) did not have a significant change (0.16–0.21%).

**Changes of Seed Weight, Oil and Protein Contents, and Fatty Acid Composition among Four Different FAD2 Genotypes.** The results of variability in seed quality traits from four FAD2 genotypes are listed in Table 3 and shown in Figure 3. The data from 2 years did not indicate much change in oil and protein contents but highly significant changes in seed weight among the four genotypes (Figure 3A). The change orders in seed weight were *aaBB* (59.73 and 60.78 g/100 seeds) > *AAbb* (51.23 and 52.54 g/100 seeds) > *aabb* (42.99 and 42.07 g/100 seeds) > *AABB* (30.08 and 34.42 g/100 seeds). The seed weight can affect the plant yield, but the seed number per plant can also contribute to the plant yield. The seed weight of the high oleic acid genotype (*aabb*) was significantly higher than the wild type (*AABB*) but also significantly lower than two single mutant genotypes (*aaBB* and *AAbb*). The results for oil and protein contents from 2 years were slightly different. Results from the

Table 3. Comparison of Seed Chemical Composition among Four *FAD2* Genotypes<sup>a</sup>

trait	year	AABB	aaBB	AAbb	aabb	MSD	range	average
protein (%)	1	23.11 a	23.11 a	23.87 a	22.55 a	2.67	22.55	23.58
	2	25.36 a	23.17 b	23.78 b	23.70 b	0.96	25.36	
oil (%)	1	46.79 a	48.59 a	47.55 a	46.54 a	2.35	46.54	47.78
	2	47.25 b	48.20 ab	49.33 a	47.95 b	1.24	49.33	
seed weight (g)	1	30.08 d	59.73 a	51.23 b	42.99 c	7.79	30.08	46.73
C16:0 (%)	2	34.42 c	60.78 a	52.54 ab	42.07 bc	10.98	60.78	
	1	13.67 a	10.27 c	11.06 b	6.26 d	0.37	6.26	10.41
C18:0 (%)	2	13.96 a	10.86 b	10.73 b	6.49 c	0.64	13.96	
	1	2.03 a	1.98 a	1.42 b	2.09 a	0.22	1.42	1.98
C18:1 (%)	2	2.11 b	2.15 b	1.57 c	2.46 a	0.20	2.46	
	1	38.05 c	46.18 b	48.04 b	80.10 a	2.90	38.05	54.47
C18:2 (%)	2	39.54 d	48.57 c	55.13 b	80.11 a	2.61	80.11	
	1	40.73 a	33.45 b	31.57 b	4.39 c	2.39	3.46	26.07
C20:0 (%)	2	38.72 a	30.63 b	25.71 c	3.46 d	2.85	40.73	
	1	1.10 b	1.16 a	0.93 c	1.14 ab	0.06	0.93	1.12
C20:1 (%)	2	1.14 b	1.23 a	0.98 c	1.30 a	0.08	1.30	
	1	1.03 c	1.60 b	1.94 a	1.83 ab	0.30	0.99	1.55
C22:0 (%)	2	0.99 d	1.45 c	1.86 a	1.69 b	0.11	1.94	
	1	2.34 c	3.20 a	2.83 b	2.69 b	0.34	2.34	2.79
C24:0 (%)	2	2.42 c	3.18 a	2.82 b	2.85 b	0.15	3.18	
	1	1.05 c	1.88 a	1.90 a	1.42 b	0.27	1.05	1.57
C26:0 (%)	2	1.14 c	1.83 a	1.83 a	1.47 b	0.10	1.90	
	1	0.00 c	0.29 a	0.33 a	0.10 b	0.08	0.00	0.22
	2	0.00 c	0.27 a	0.30 a	0.16 b	0.04	0.33	

<sup>a</sup>Four genotypes were AABB, aaBB, AAbb, and aabb. Minimum significant difference (MSD), seed protein, oil, and methyl esters (%), and seed weight (g/100 seeds). If followed by the same letter after the trait value in each row, there were no statistically significant differences between two or more genotypes.

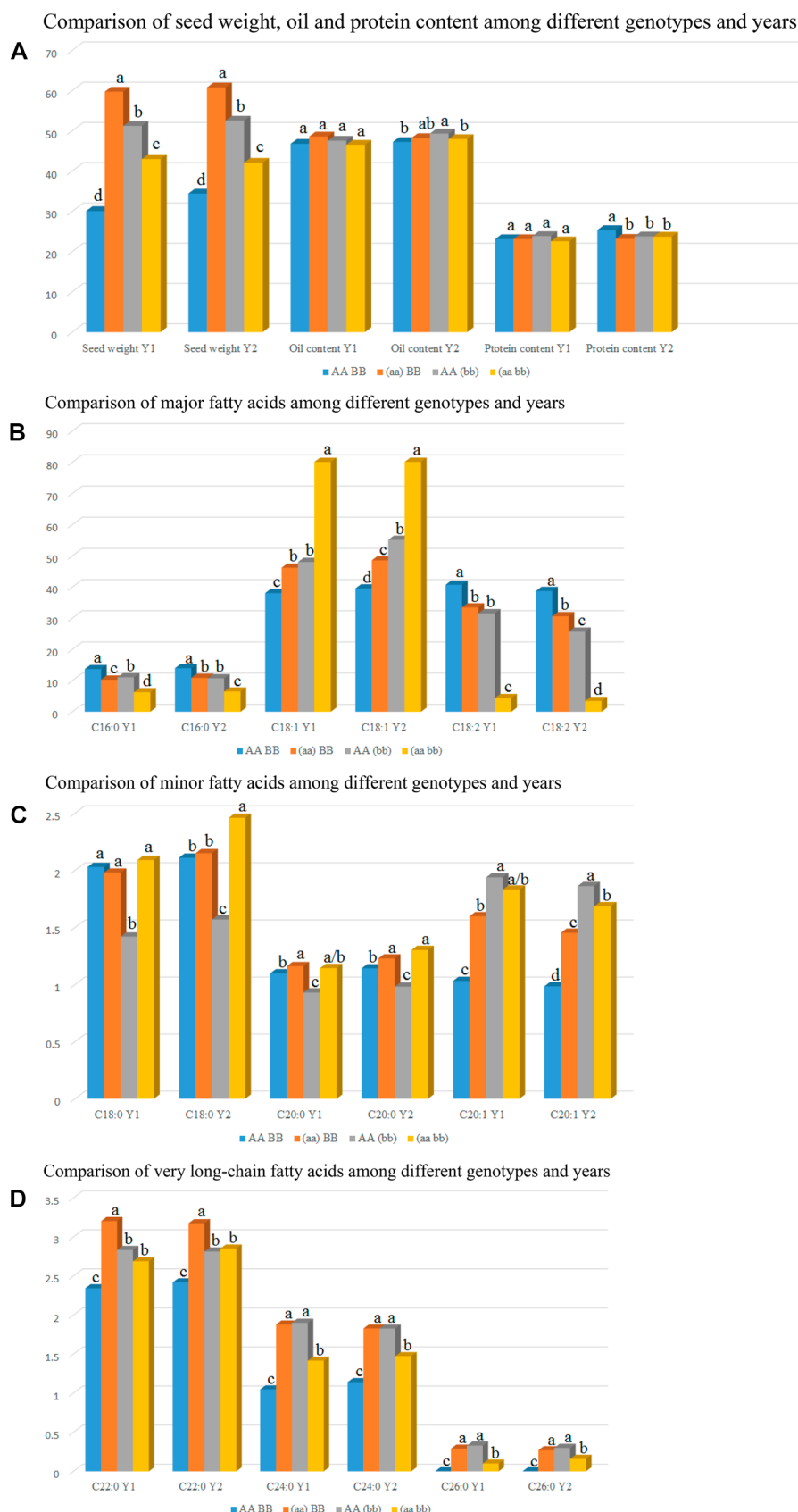
first year showed no significant differences in oil and protein contents among four genotypes. For the second year, the oil content of AAbb (49.33%) was slightly higher than the oil content of AABB (47.25%) and aabb (47.95%). The protein content of AABB (25.36%) was slightly higher than three other genotypes of aabb (23.17%), AAbb (23.78%), and aabb (23.70%). For the differences in oil and protein contents among the four genotypes from the second year, they were statistically significant, but the difference value was only 1–2%. Because the results of oil and protein contents from 2 years were not consistent, further experiments are needed. Similar results were also observed in double mutant soybean (*FAD2-1aabb*). The level of oleic acid was enhanced to 80%, whereas the oil and protein contents were not significantly changed.<sup>14</sup>

For major fatty acids (Table 3 and Figure 3B), high oleate double mutant (*aabb*) had a significantly lower amount of palmitic acid (6.26 and 6.49%) than the other three genotypes (*aaBB*, 10.27 and 10.86%; *AAbb*, 11.06 and 10.73%; and *AABB*, 13.67 and 13.96%). As expected, high oleate double mutant (*aabb*) had the highest and lowest levels of oleic and linoleic acids (80.1 and 80.11% and 4.39 and 3.46%) among the four genotypes (*aaBB*, 46.18 and 48.57% and 33.45 and 30.63%; *AAbb*, 48.04 and 55.13% and 33.45 and 30.63%; and *AABB*, 38.05 and 39.54% and 40.73 and 38.72%, respectively). The level of oleate enhancement in the double mutant mainly came from the decrease of linoleic acid but also from the decrease of palmitic acid. Similar results were also observed in double mutant soybean (*FAD2-1aabb*). In comparison to wild type soybean (*FAD2-1AABB*), the enhanced level of oleic acid in the double mutant was mainly from the decrease of palmitic acid (from 12.3 to 7.9%) and linoleic acid (from 54.6 to 4.2%).<sup>14</sup> Additionally, as shown in the second year results, the levels of oleic and linoleic acids (55.13 and 25.71%)

from single mutant *AAbb* was significantly higher and lower than the those from single mutant *aaBB* (48.57 and 30.63%). The differences in the levels of oleic and linoleic acids between single mutant *aaBB* and *AAbb* may be explained by two reasons. One was that the insertion mutation in *FAD2B* may have a larger effect on reducing desaturase activity than the substitution mutation in *FAD2A*. Another was that initially *FAD2B* was expressed in a higher level than *FAD2A* in the wild type (*AABB*) during seed development.

For minor fatty acids (Table 3 and Figure 3C), the high oleate double mutant (*aabb*) had higher amounts of stearic and arachidic acids (2.09 and 2.46% and 1.14 and 1.30%) than other genotypes, especially much higher than the single mutant *AAbb* (1.42 and 1.57% and 0.93 and 0.98%). The high oleate double mutant also had a higher amount of gadoleic acid (1.83 and 1.69%) than wild type *AABB* (1.03 and 0.99%) and single mutant *aaBB* (1.60 and 1.45%) but slightly lower than or similar to the single mutant *AAbb* (1.94 and 1.86%). The results for the minor fatty acids from 2 years were consistent. For very long-chain fatty acids (Table 3 and Figure 3D), single mutant *aaBB* contained a significantly higher amount of behenic acid (3.20 and 3.18%) than the three other genotypes (*AAbb*, 2.83 and 2.82%; *aabb*, 2.69 and 2.85%; and *AABB*, 2.34 and 2.42%). Two single mutants (*aaBB* and *AAbb*) contained significantly higher amounts of lignoceric acid (1.88 and 1.83% and 1.90 and 1.83%) and hexacosanoic acid (0.29 and 0.27% and 0.33 and 0.30%) than the wild type *AABB* (1.05 and 1.14% and 0.00 and 0.00%) and double mutant *aabb* (1.42 and 1.47% and 0.10 and 0.16%). The results for very long-chain fatty acids among four different genotypes from 2 years were also very consistent.

**Correlations between Investigated Seed Quality Traits.** The correlations between 12 investigated seed quality traits are



**Figure 3.** Comparison of seed quality traits among different *FAD2* genotypes for 2 years: (A) comparison of seed weight and oil and protein contents, (B) comparison of major fatty acids, (C) comparison of minor fatty acids, and (D) comparison of very long-chain fatty acids.

shown in Table 4 (but only the correlation with  $r \geq 0.8$  at  $p < 0.0001$  was mentioned in the text). Palmitic acid was positively

correlated with linoleic acid ( $r = 0.95$  at  $p < 0.0001$ ) but negatively correlated with oleic acid ( $r = -0.95$  at  $p < 0.0001$ ). Stearic acid

**Table 4.** Pearson Correlation Coefficients, Probability, and Number of Observations for Oil Content, Seed Weight, and Fatty Acid Composition

	protein	SdWt <sup>a</sup>	C16:0	C18:0	C18:1	C18:2	C20:0	C20:1	C22:0	C24:0	C26:0
oil	0.11	0.69	−0.04	0.12	0.10	−0.06	−0.06	−0.36	−0.57	−0.27	0.06
	0.3509	<0.0001	0.7643	0.2963	0.3967	0.6018	0.6030	0.0012	<0.0001	0.0164	0.5780
	77	77	77	77	77	77	77	77	77	77	77
protein	1	−0.04	0.24	0.02	−0.13	0.13	−0.02	−0.27	−0.24	−0.20	−0.06
		0.760580	0.0304	0.8826	0.2458	0.2694	0.8324	0.0161	0.0333	0.0690	0.6060
			80	80	80	80	80	80	80	80	80
SdWt	1		−0.13	−0.05	0.07	−0.03	−0.07	−0.03	0.13	0.25	0.44
			0.2434	0.6721	0.5643	0.7625	0.5407	0.7841	0.2398	0.0285	<0.0001
			80	80	80	80	80	80	80	80	80
C16:0		1		−0.28	−0.95	0.95	−0.35	−0.56	−0.23	−0.21	−0.24
				0.0124	<0.0001	<0.0001	0.0013	<0.0001	0.0434	0.0614	0.0348
				80	80	80	80	80	80	80	80
C18:0			1		0.35	−0.36	0.91	−0.48	−0.22	−0.48	−0.48
					0.0013	0.0012	<0.0001	<0.0001	0.0500	<0.0001	<0.0001
					80	80	80	80	80	80	80
C18:1				1		−0.99	0.35	0.40	−0.01	−0.03	0.02
						<0.0001	0.0015	0.0002	0.9438	0.7762	0.8341
						80	80	80	80	80	80
C18:2					1		−0.37	−0.43	−0.04	0.00	−0.04
							0.0008	<0.0001	0.7579	0.9724	0.7275
							80	80	80	80	80
C20:0						1		−0.26	0.14	−0.21	−0.26
								0.0201	0.2073	0.052	0.0180
								80	80	80	80
C20:1							1		0.69	0.79	0.71
									<0.0001	<0.0001	<0.0001
									80	80	80
C22:0								1		0.83	0.61
										<0.0001	<0.0001
										80	80
C24:0										1	0.90
											<0.0001
											80

<sup>a</sup>SdWt = seed weight (g/100 seeds).

was positively correlated with arachidic acid ( $r = 0.91$  at  $p < 0.0001$ ). Oleic acid was negatively correlated with linoleic acid ( $r = -0.99$  at  $p < 0.0001$ ). Gadoleic and behenic acids were positively correlated with lignoceric acid ( $r = 0.79$  and  $0.83$  at  $p < 0.0001$ ). Lignoceric acid was positively correlated with hexacosanoic acid ( $r = 0.90$  at  $p < 0.0001$ ). These highly significant correlations were consistent with the results from our previous study in peanut.<sup>11</sup> The correlation information may help breeders to understand how other traits will be changed or affected while they focus on improving specific traits (such as increasing or decreasing the amount of a specific biochemical).

In summary, as peanut seeds matured, seed chemical composition changed at different rates for the different traits investigated. The oil content, seed weight, oleic acid (C18:1), and arachidic acid (C20:0) significantly increased, whereas linoleic acid (C18:2), gadoleic acid (C20:1), behenic acid (C22:0), and lignoceric acid (C24:0) significantly decreased. The protein content, palmitic acid (C16:0), stearic acid (C18:0), and hexacosanoic acid (C26:0) did not change significantly during the investigated maturation process. Among the four *FAD2* genotypes, genotype *aabb* with about 80% oleic acid is the desired trait for new peanut cultivar development, product processing, and human consumption. We observed that enhancing the oleic acid level also significantly decreased levels of linoleic and palmitic acids but did

not significantly change oil and protein contents (two important peanut quality traits). The results from the 2 years were generally consistent. The information revealed from this study will be useful for peanut research and industry.

## AUTHOR INFORMATION

### Corresponding Author

\*Telephone: 001-770-229-3342. Fax: 001-770-229-3323.  
E-mail: [mingli.wang@ars.usda.gov](mailto:mingli.wang@ars.usda.gov).

### ORCID

Ming Li Wang: [0000-0001-9406-8951](https://orcid.org/0000-0001-9406-8951)

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The authors declare no competing financial interest.

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